CLAIMS

1. A method, comprising:

a) providing: i) a misaminoacylated initiator tRNA molecule which only recognizes the first AUG codon that serves to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; and

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker, said affinity marker and said C-terminal marker.

2. The method of Claim 1, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

3. The method of Claim 1, wherein the translation system comprises a cellular or cell-free translation system.

4. The method of Claim 3, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells *in vivo*, isolated immortalized cells, human cells and combinations thereof.

5. The method of Claim 3, wherein the cell-free translation system is selected from the group consisting of *Escherichia coli* lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and

combinations thereof.

6. The method of Claim 3, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

7. The method of Claim 3, wherein the cell-free translation system is a continuous flow or dialysis system.

8. The method of Claim 1, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

9. The method of Claim 3, wherein said nascent protein is functionally active.

10. The method of Claim 1, wherein said first marker comprises a fluorescent compound.

11. The method of Claim 10, wherein said fluorescent compound is selected from dipyrromethene boron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

12. The method of Claim 1, wherein said C-terminal comprises a histidine tag.

13. A method, comprising:

a) providing i) a misaminoacylated initiator tRNA molecule which only recognizes the first AUG codon that serves to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; and

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker at the N-terminus of said protein, a C-terminal marker, and said affinity marker adjacent to said first marker.

14. The method of Claim 13, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

15. The method of Claim 13, wherein the translation system comprises a cellular or cell-free translation system.

16. The method of Claim 15, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells *in vivo*, isolated immortalized cells, human cells and combinations thereof.

17. The method of Claim 15, wherein the cell-free translation system is selected from the group consisting of *Escherichia coli* lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

18. The method of Claim 15, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

19. The method of Claim 15, wherein the cell-free translation system is a continuous flow or dialysis system.

20. The method of Claim 13, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

21. The method of Claim 13, wherein said nascent protein is functionally active.

22. The method of Claim 13, wherein said first marker comprises a fluorescent compound.

23. The method of Claim 22, wherein said fluorescent compound is selected from dipyrromethene boron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

24. The method of Claim 13, wherein said C-terminal comprises a histidine tag.

25. A method, comprising:

a) providing i) a misaminoacylated tRNA molecule which only recognizes the first codon designed to serve to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; and

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker, said affinity marker and said C-terminal marker.

26. The method of Claim 25, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones,

immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

27. The method of Claim 25, wherein the translation system comprises a cellular or cell-free translation system.

28. The method of Claim 27, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells, cells *in vivo*, isolated immortalized cells, human cells and combinations thereof.

29. The method of Claim 27, wherein the cell-free translation system is selected from the group consisting of *Escherichia coli* lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

30. The method of Claim 27, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

31. The method of Claim 27, wherein the cell-free translation system is a continuous flow or dialysis system.

32. The method of Claim 25, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

33. The method of Claim 25, wherein said nascent protein is functionally active.

34. The method of Claim 25, wherein said first marker comprises a fluorescent compound.

35. The method of Claim 34, wherein said fluorescent compound is selected from dipyrrometheneboron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

36. The method of Claim 25, wherein said C-terminal comprises a histidine tag.

37. A method, comprising:

a) providing i) a misaminoacylated tRNA molecule which only recognizes the first codon designed to serve to initiate protein synthesis, said misaminoacylated initiator tRNA molecule comprising a first marker, and ii) a nucleic acid template encoding a protein, said protein comprising an affinity marker and a C-terminal marker; and

b) introducing said misaminoacylated initiator tRNA to a translation system comprising said template under conditions such that a nascent protein is generated, said protein comprising said first marker at the N-terminus of said protein, a C-terminal marker, and said affinity marker adjacent to said first marker.

38. The method of Claim 38, wherein the nascent protein generated is selected from recombinant gene products, gene fusion products, enzymes, cytokines, carbohydrate and lipid binding proteins, nucleic acid binding proteins, hormones, immunogenic proteins, truncated proteins, mutant proteins, human proteins, viral proteins, bacterial proteins, parasitic proteins and fragments and combinations thereof.

39. The method of Claim 38, wherein the translation system comprises a cellular or cell-free translation system.

40. The method of Claim 39, wherein the cellular translation system is selected from prokaryotic cells, eukaryotic cells, tissue culture cells, primary cells,

cells *in vivo*, isolated immortalized cells, human cells and combinations thereof.

41. The method of Claim 39, wherein the cell-free translation system is selected from the group consisting of *Escherichia coli* lysates, wheat germ extracts, insect cell lysates, rabbit reticulocyte lysates, frog oocyte lysates, dog pancreatic lysates, human cell lysates, mixtures of purified or semi-purified translation factors and combinations thereof.

42. The method of Claim 39, wherein said cell-free translation system is incubated at a temperature of between about 25°C to about 45°C.

43. The method of Claim 39, wherein the cell-free translation system is a continuous flow or dialysis system.

44. The method of Claim 37, wherein the tRNA molecule is aminoacylated by chemical or enzymatic misaminoacylation.

45. The method of Claim 37, wherein said nascent protein is functionally active.

46. The method of Claim 37, wherein said first marker comprises a fluorescent compound.

47. The method of Claim 46, wherein said fluorescent compound is selected from dipyrromethene boron difluoride (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) dyes and derivatized coumarin.

48. The method of Claim 37, wherein said C-terminal comprises a histidine tag.